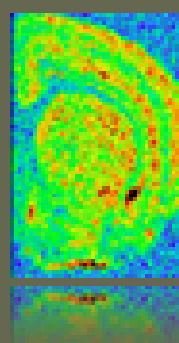




Structural Modification and Biological Evaluation of Dmt¹-DALDA Analogues



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Introduction

The highly charged tetrapeptide Dmt-DALDA (Dmt-D-Arg-Phe-Lys-NH₂) has been previously identified as a potent μ -opioid receptor agonist and serves as a lead compound for the further development of novel therapeutic (peptidic) opioid analgesics.^[1] Structural modifications of the peptide have been performed in order to determine the role of the side chain charges, N-methylation of the Phe³-Lys⁴ amide bond, and the influence of a benzazepine conformational constraint^[2] at the level of Phe³.

All prepared compounds have been tested for their *in vitro* affinity and activity (GPI and MVD assays) and four of them for their *in vivo* tissue distribution and *in vitro* permeability (in- and efflux into and out of mouse brain and caco-2 test)

Activity : μ agonist and δ agonist

Peptide	GPI (IC ₅₀ , nM)	Rel.pot.*	MVD (IC ₅₀ , nM)	Rel.pot.*	MOR (IC ₅₀ , nM)	Rel.binding*	DOR (IC ₅₀ , nM)	Rel.binding*
H-Dmt-D-Arg-Phe-Lys-NH ₂ 1	1.41	81	23.1	2.7	0.58	147	877	0.12
H-Dmt-D-Arg-Phe- Nle -NH ₂ 2	2.2	119	6.2	2.6	0.23	132.5	15.2	0.64
H-Dmt- D-Cit -Phe-Lys-NH ₂ 3	0.31	791	1.4	9.64	0.57	52.4	50.0	0.25
H-Dmt- D-Cit -Phe- Nle -NH ₂ 4	0.89	203	4.7	4.12	0.79	28.1	3.98	1.46
H-Dmt-D-Arg-Phe- NMe Lys-NH ₂ 5	8.3	106	7.0	6.0	0.26	115.4	309	0.027
H-Dmt-D-Arg-Phe- NMeNle -NH ₂ 6	4.35	45	1.5	9.3	0.26	135.8	16.0	0.51
H-Dmt- D-Cit -Phe- NMe Lys-NH ₂ 7	1.01	398	4.4	9.5	0.94	23.6	39.2	0.347
H-Dmt- D-Cit -Phe- NMeNle -NH ₂ 8	0.65	434	1.5	1.0	2.92	11.3	5.03	1.94
H-Dmt-D-Arg- Aba -Lys-NH ₂ 9	76.0	13	64 (IC ₂₅ , max50%)	0.67	0.605	50.97	38.56	0.276
H-Dmt-D-Arg- Aba-Nle -NH ₂ 10	1.45	327	3.1	5	0.432	82.28	7.46	1.24
H-Dmt- D-Cit-Aba -Lys-NH ₂ 11	49.0	18.9	1000	30 % inh	2.618	8.547	56.91	0.108
H-Dmt- D-Cit-Aba-Nle -NH ₂ 12	0.78 (IC _{35max} : 67 %)	643	1.16	17.5	0.931	38.64	3.176	2.1816
H-Dmt-D-Arg- Aba-Gly -NH ₂ 13	0.32	/	0.42	/	0.15	/	0.60	/

*relative to Leu⁵-enkephalin (binding : MOR: 29.9 nM, DOR: 9.77 nM)

side-chain charges are not essential for in vitro activity

N-methylation is allowed for in vitro activity and potentially stabilizes the amide bond between Phe³ and Lys⁴

the conformational constraint of the Phe residue by the Aba results in highly potent compounds, but is not compatible with the Lys side chain

Superpotent μ/δ agonist

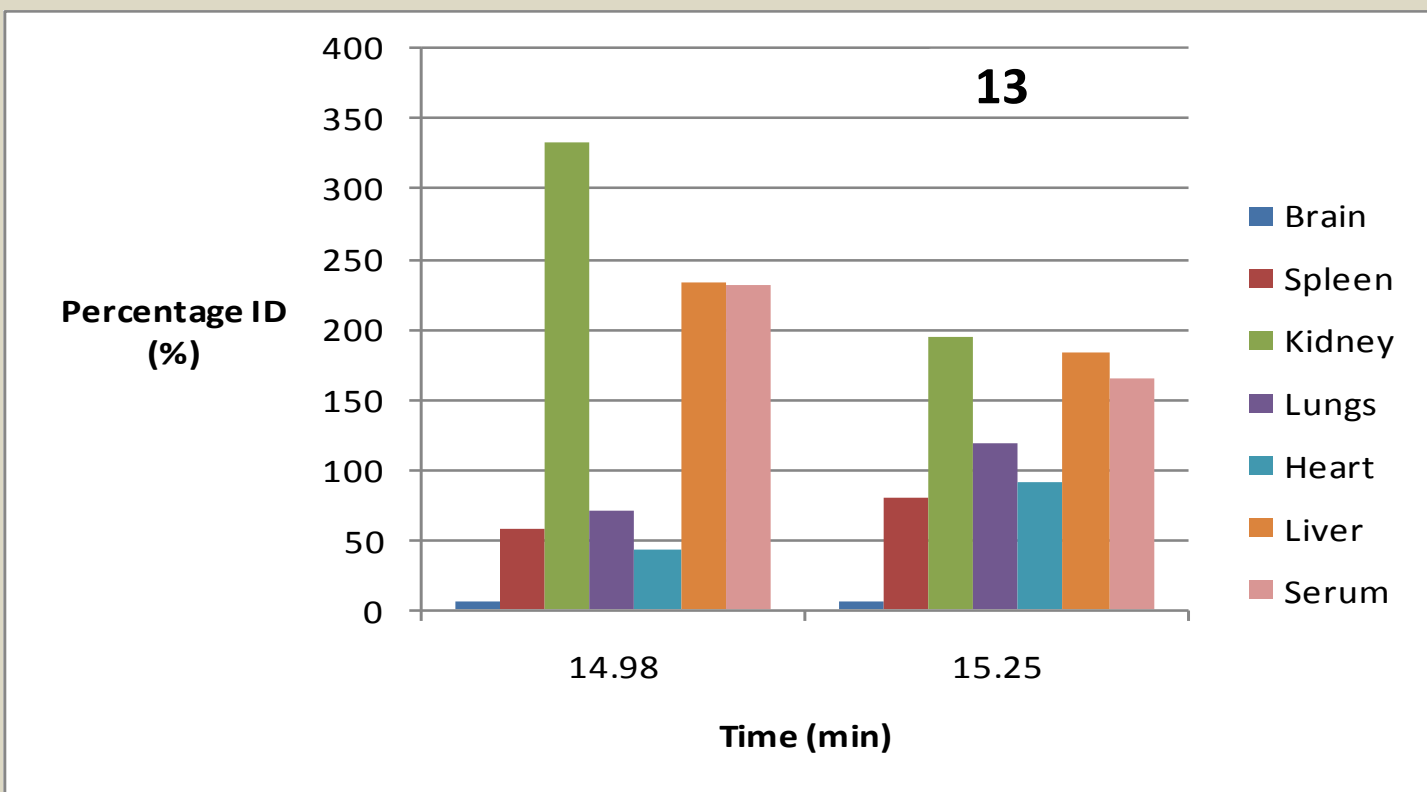
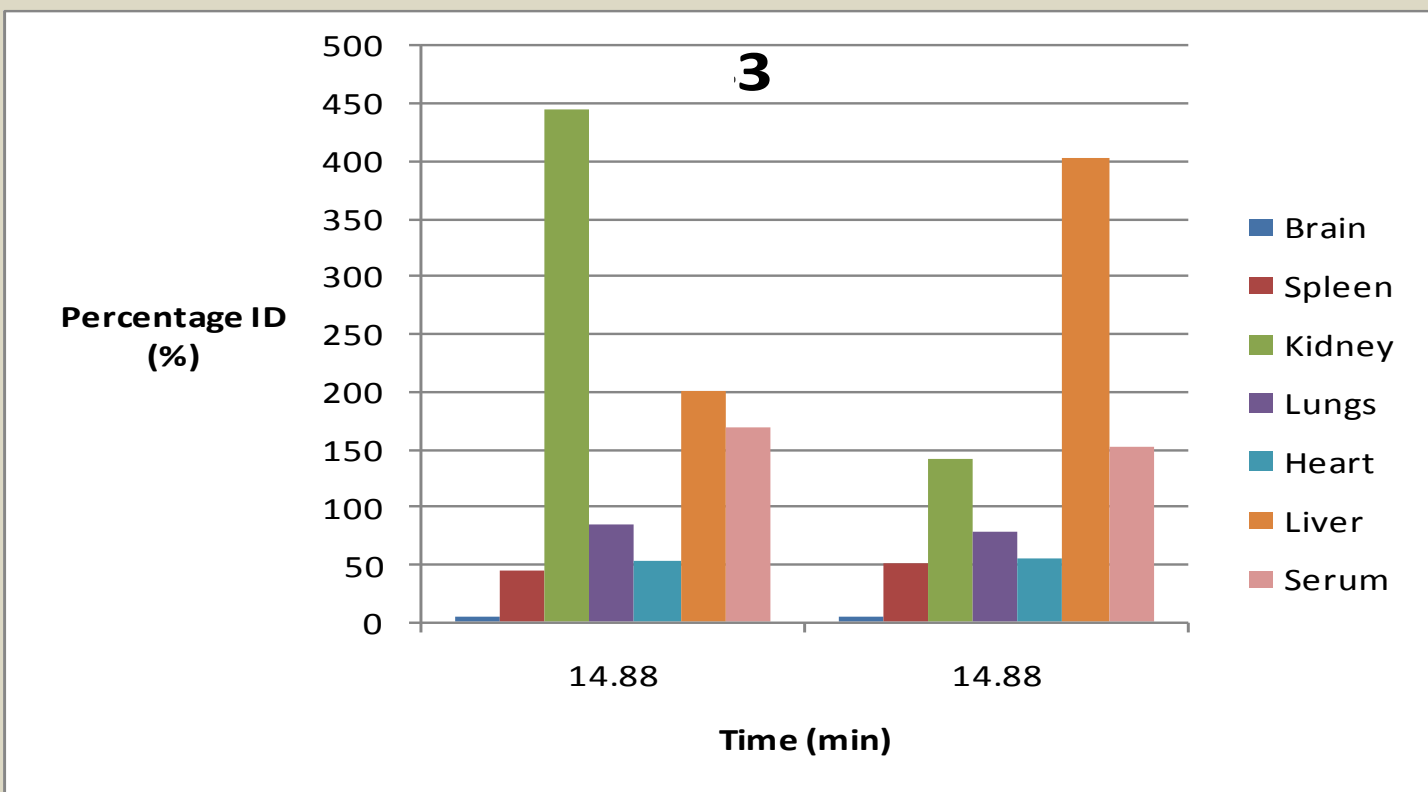
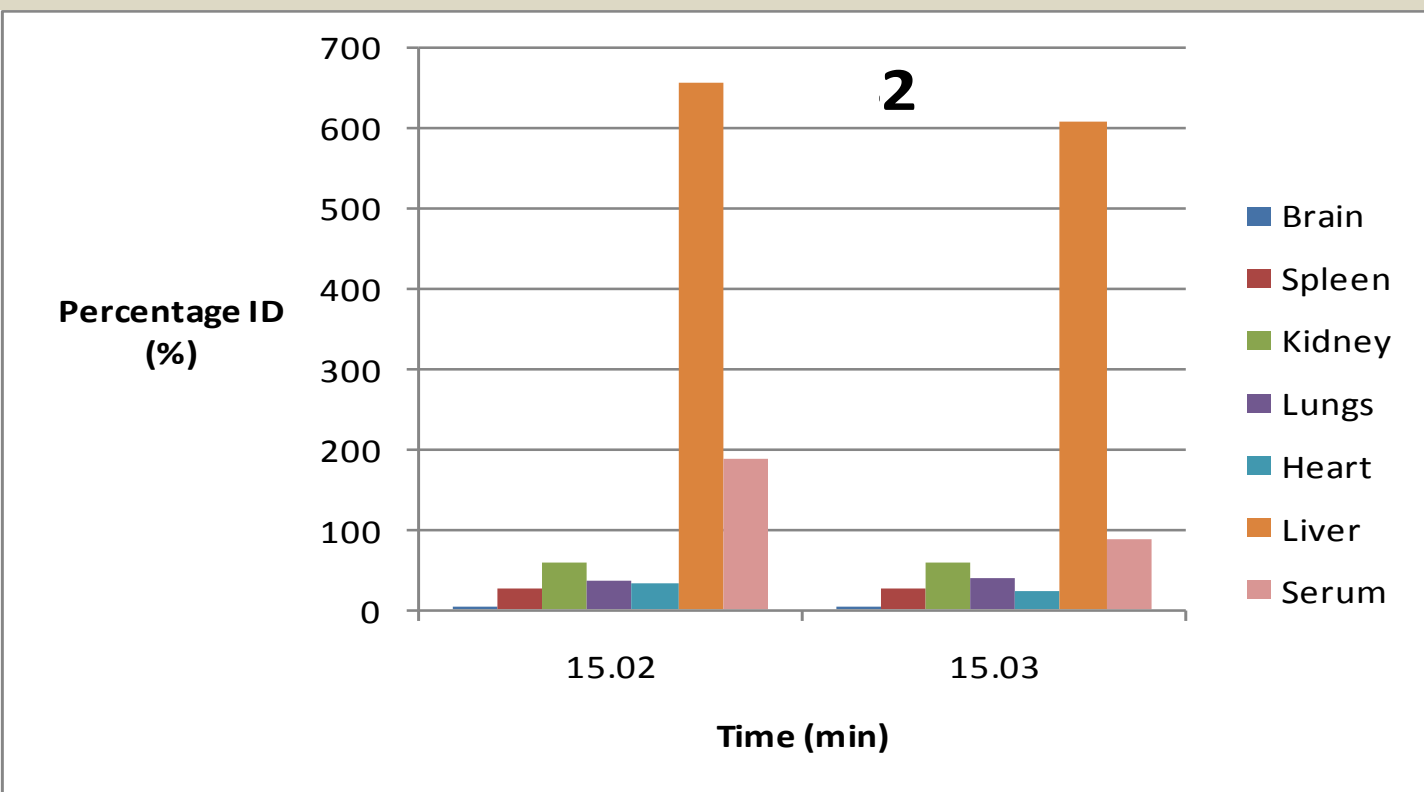
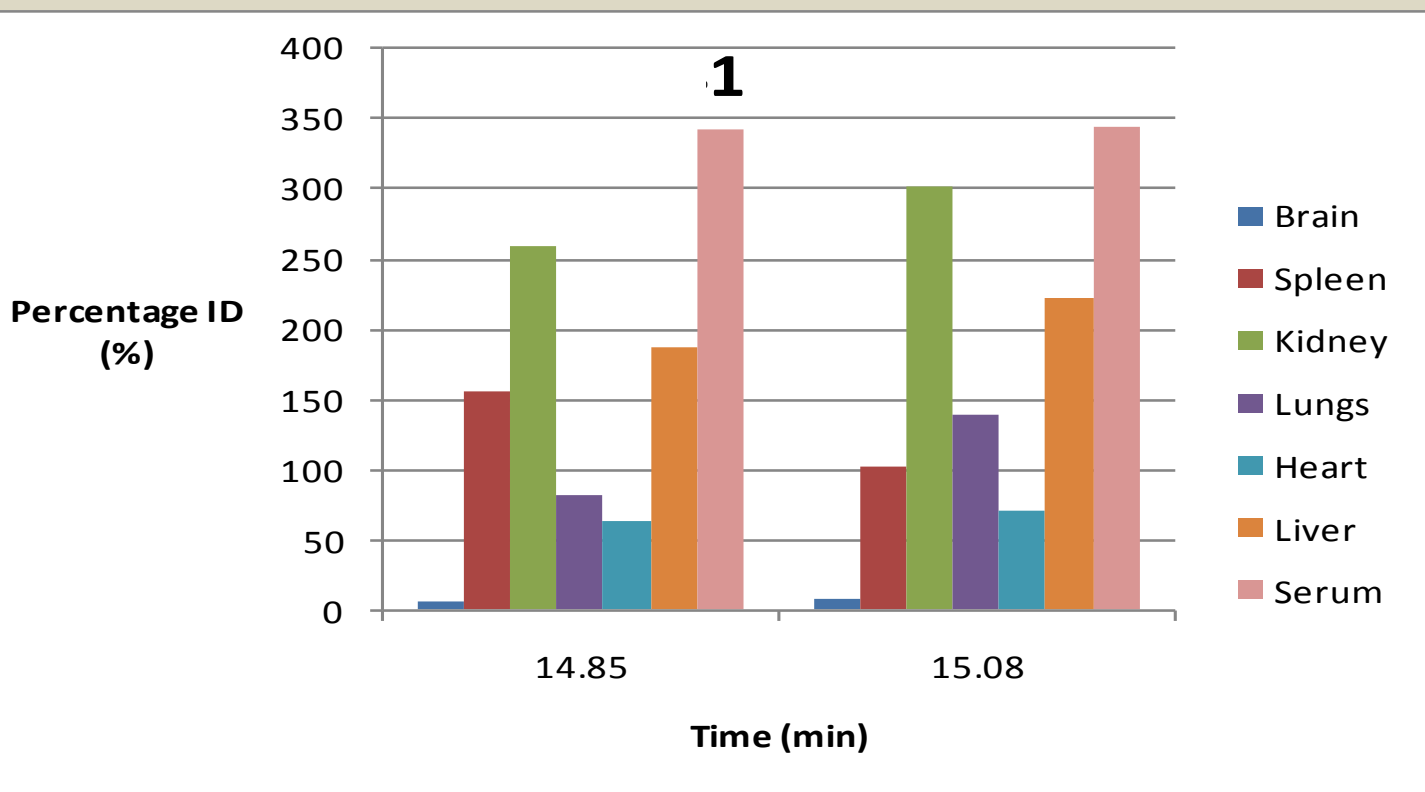
Permeability

BBB penetration of ¹²⁵I-peptides^[3]

Peptide	K _{in} (μl/(g×min))	Capillary fraction (%)	Parenchyma fraction (%)	k _{out} (min ⁻¹)
H-Dmt-D-Arg-Phe-Lys-NH ₂ 1	0.37 ± 0.08	23.12 ± 10.26	76.88 ± 10.26	0.058 ± 0.11
H-Dmt-D-Arg-Phe- Nle -NH ₂ 2	0.25 ± 0.25	32.97 ± 9.99	67.03 ± 9.99	0.039 ± 0.11
H-Dmt- D-Cit -Phe-Lys-NH ₂ 3	-0.31 ± 0.45	19.39 ± 15.81	80.61 ± 15.81	0.027 ± 0.022
H-Dmt-D-Arg- Aba-Gly -NH ₂ 13	0.45 ± 0.20	30.95 ± 19.75	69.05 ± 19.75	0.096 ± 0.046

- Significant influx for peptide 1,2 and 13
- No efflux for peptides 1 and 2, while statistically significant efflux observed for 13

Relative concentrations in the different tissues : MTR experiment

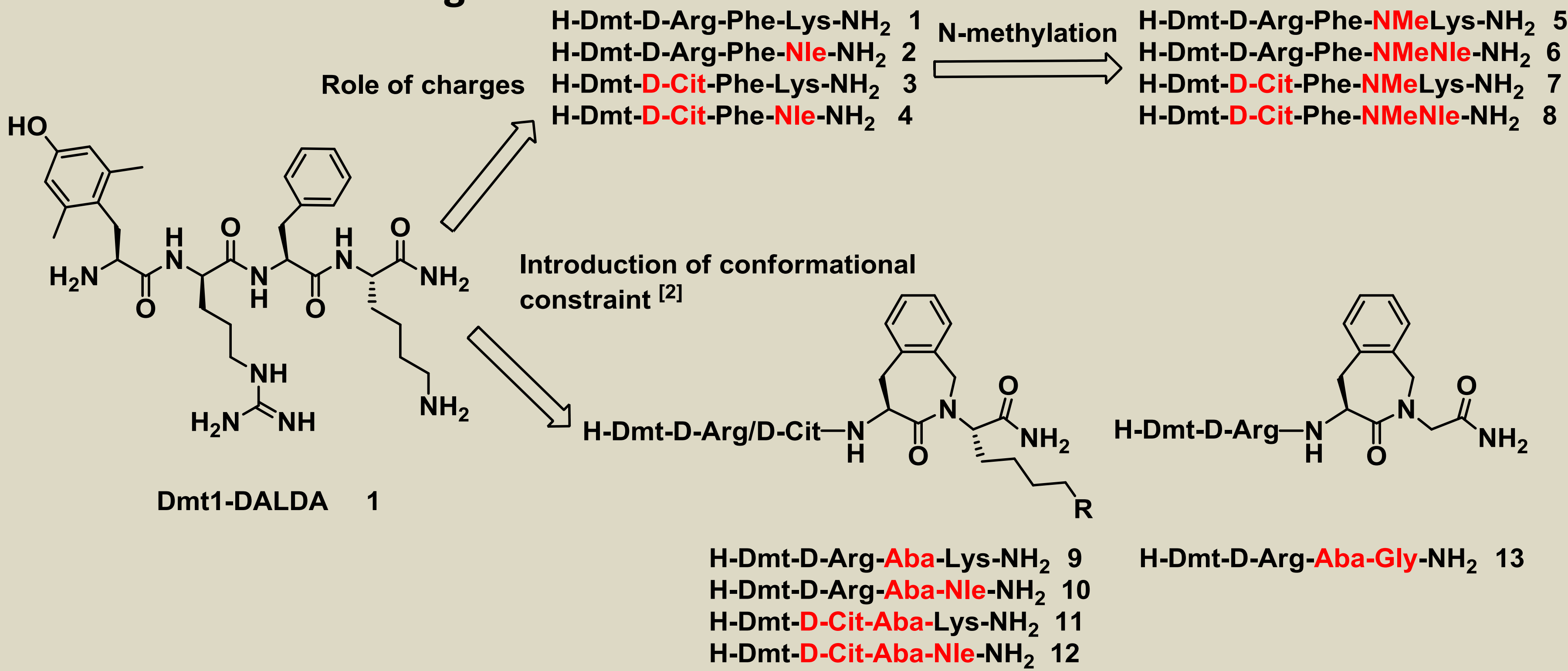


- Significative accumulation in liver for H-Dmt-D-Arg-Phe-**Nle**-NH₂ **2**
- No specific accumulation for the three others peptides

References

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b) Van Rompaey K., Van den Eynde I., De Kimpe N., Tourwe D., *Tetrahedron*, **2003**, *59*, p 4421-4432
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Dmt¹-DALDA and analogues



CACO-2 test

Peptide	Chem. Properties MW / Log P / Log D (7.4)/Log S tPSA / nD / nA / nRot	A-B perm. (10 ⁻⁶ cm/s)	A-B recover (%)	B-A perm. (10 ⁻⁶ cm/s)	B-A recover (%)
H-Dmt-D-Arg-Phe-Lys-NH ₂ 1	639.8 / 0.4 / -0.4 / -6.6 264.6 / 10 / 8 / 20	<0.1	89	<0.1	95
H-Dmt-D-Arg-Phe- Nle -NH ₂ 2	624.8 / 1.9 / 1.1 / -7.0 238.5 / 9 / 7 / 19	<0.4	101	<0.5	89
H-Dmt- D-Cit -Phe-Lys-NH ₂ 3	640.8 / 0.4 / -0.4 / -7.4 257.8 / 9 / 8 / 19	<0.6	85	<0.8	111

the CACO-2 assay predicts a limited transport through the intestinal membrane

Conclusions

- Side chain charges and/or N-methylation are not essential for in vitro affinity and activity
- Significant influx into the mouse brain for peptide 1, 2 and 13 demonstrated by the K_{in} values and the parenchyma fraction
- The CACO-2 test predict a limited transport through the intestinal membrane (GI → blood).

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